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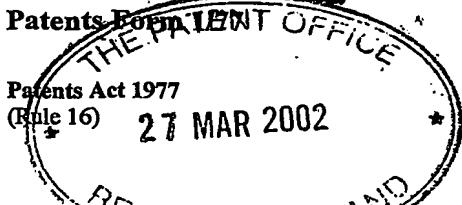
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Request for grant of a patent

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1. Your reference	1-32411P1/FMI		
2. Patent application number (The Patent Office will fill in this part)	0207224.7 27 MAR 2002		
3. Full name, address and postcode of the or of each applicant (underline all surnames)	NOVARTIS FORSCHUNGSSTIFTUNG, ZWEIGNIEDERLASSUNG FRIEDRICH MIESCHER INSTITUTE FOR BIOMEDICAL RESEARCH, MAULBEERSTRASSE 66, CH-4058 BASEL, SWITZERLAND		
Patent ADP number (if you know it) If the applicant is a corporate body, give the country/state of its incorporation SWITZERLAND 8206583001			
4. Title of invention	Tenascin-W Compositions and uses thereof		
Name of your agent (if you have one) "Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode) B.A. YORKE & CO. CHARTERED PATENT AGENTS COOMB HOUSE, 7 ST. JOHN'S ROAD ISLEWORTH MIDDLESEX TW7 6NH			
Patents ADP number (if you know it) 1800001			
6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number	Country	Priority application number (if you know it)	Date of filing (day/month/year)
7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application		Date of filing (day/month/year)
8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if: a) any applicant named in part 3 is not an inventor, or b) there is an inventor who is not named as an applicant, or c) any named applicant is a corporate body. (see note (d))	Yes		

Patents Form 1/77

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Description **37**

Claim(s) **8**

Abstract **1**

Drawing(s)

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (*Patents Form 7/77*)

Request for preliminary examination and search (*Patents Form 9/77*)

Request for substantive examination (*Patents Form 10/77*)

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Sequence listing pages 38-67

11.

I/We request the grant of a patent on the basis of the following invention

Signature

Date

B.A. Yorke

B.A. Yorke & Co.

27 March 2002

12. Name and daytime telephone number of person to contact in the United Kingdom

Mrs. E. Cheetham

020 8560 5847

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TENASCIN-W COMPOSITIONS AND USES THEREOF

5 The present invention relates to compositions being specifically expressed in tumours, to active agents having anti-tumour and/or anti-tumourigenic activity and to agents having the ability of changing a condition ameliorated by promotion of stem cell differentiation, in particular osteoblast formation, such as in osteogenesis, pharmaceutical compositions of these agents and the

10 pharmaceutical uses of such agents and compositions. The invention also relates to *in vitro* methods of screening agents for anti-tumour and/or anti-tumourigenic activity as well as for agents ameliorating the promotion of stem cell differentiation, in particular osteoblast formation, such as in osteogenesis.

15 The adherence of cells to each other and to the extracellular matrix (ECM), as well as the cellular signals transduced as a consequence of such binding, are of fundamental importance to the development and maintenance of body form and function. The ECM has an ~~important~~ regulatory function in tissue homeostasis and, together with oncogenes and tumour suppressor genes is

20 critically involved in tumourigenesis (reviewed in Boudreau, N. & Bissell, M. J. (1998) *Curr Opin Cell Biol* 10: 640-646 and Ruoslahti, E. (1999) *Adv Cancer Res* 76: 1-20).

In the more affluent countries of the world cancer is the cause of death of

25 roughly one person in five. The American Cancer Society in 1993 reported that the five most common cancers are those of the lung, stomach, breast, colon/rectum and the uterine cervix. Tumors of this type often metastasize through lymphatic and vascular channels. Cancer is not fatal in every case and only about half the number of people who develop cancer die of it. The

30 problem facing cancer patients and their physicians is that seeking to cure cancer is like trying to get rid of weeds. One way to treat cancer effectively is to get an early diagnosis. Most cancers are not extensively vascularized (and therefore not invasive) during the early stages of development. The transition to a highly vascularized, invasive and ultimately metastatic cancer which

spreads throughout the body commonly takes ten years or longer. If the cancer is detected prior to invasion, surgical removal of the cancerous tissue is an effective cure. However, cancer is often detected only upon manifestation of clinical symptoms. Generally, such symptoms are present 5 only when the disease is well established, often after metastasis has occurred, and the prognosis for the patient is poor, even after surgical resection of the cancerous tissue. Early detection of cancer therefore is important in that detection may significantly reduce its morbidity. A reliable, non-invasive, and accurate technique for diagnosing cancer at an early stage 10 would help save many lives.

However, although cancer cells can be removed surgically or destroyed with toxic compounds or with radiation, it is very hard to eliminate all of the cancerous cells. Apart from early diagnosis, a general goal is to find better 15 ways of selectively killing cancer cells whilst leaving normal cells of the body unaffected. Part of that ~~effort~~ involves identifying new anti-cancer agents.

Apart from ~~turning~~, the ECM has an important regulatory function in 20 tissue homeostasis and in the development and maintenance of body form and function, e.g. in the development or remodeling of skeleton or in bone morphogenesis. Bone marrow has stem cells with osteogenic potential and is made up of determined osteogenic precursor cells that are committed to osteogenesis and of inducible osteogenic precursor cells. Determined 25 osteogenic precursor cells can differentiate into bone without an exogenous signal. Inducible osteogenic precursor cells require a molecular signal for initiating the differentiation program, e.g. induced by binding to extracellular matrix.

A number of molecules mediating cell adhesion have been identified and 30 characterized at the molecular level both in vertebrates and invertebrates. Tenascins are a family of large multimeric extracellular matrix proteins, each consisting of homologous subunits built from variable numbers of repeated

is also found in fibrinogens. Tenascin-C was the first member of the family to be discovered in one instance as a myotendinous antigen (Chiquet, M. & Fambrough, DM. (1984) *J Cell Biol* 98(6):1937-1946) and in another, as a protein enriched in the stroma of gliomas (Bourdon, MA. et al (1983) *Cancer Res* 43(6):2796-2805). These discoveries already reflect the major sites of tenascin-C expression, namely tendons and ligaments and the extracellular matrix of tumor stroma. A further instance of the discovery of tenascin-C, also termed hexabrachion reflects its interaction with fibronectin (Erickson, HP. et al. (1984) *Nature* 311(5983):267-9). Enforced interaction of tumour cells with fibronectin can block proliferation in cell culture and can decrease tumour growth in nude mice (Akamatsu H. et al (1996) *Cancer Res* 56: 4541-4546 and Giancotti, F. G & Ruoslahti, E. (1990) *Cell* 60: 849-859). Tenascin-C was shown to disrupt the interaction of cells with fibronectin and in this manner may enhance tumour cell proliferation. Chiquet-Ehrismann, R. et al (1988) *Cell* 53: 383-390 were the first to show that tenascin-C binds to fibronectin, blocks cell attachment to fibronectin and increases proliferation of rat breast carcinoma cells (Chiquet-Ehrismann, R. et al (1986) *Cell* 47: 131-139).

Each tenascin family member exhibits a specific gene expression pattern during embryogenesis and in the adult (for review see Chiquet-Ehrismann, R. (1995) *Experientia* 51(9-10):853-62). Tenascin-R is an extracellular matrix component of the nervous system found mainly in brain tissue (Pasheva, P. et al. (2001) *Prog Brain Res.* 132:103-14. Review), whereas tenascin-X is prominently expressed in muscle and skin connective tissue. In one patient, tenascin-X deficiency has been reported to result in an Ehler's Danlos phenotype (Burch, GH. et al. (1997) *Nat Genet* 17(1):104-8). Tenascin-C is present in a large number of developing tissues including the nervous system. It is abundant in mature ligaments and tendons but is absent from skeletal and heart muscle, unless the muscle has been injured. Tenascin-C expression is elevated in essentially all carcinomas as well as in many other types of tumors (for review see Chiquet-Ehrismann, R. (1993) *Semin Cancer Biol* 4(5):301-10). Furthermore, tenascin-C is upregulated in wound healing (Latijnhouwers, MA. et al. (1996) *J Pathol* 178(1):30-5), during skeletogenesis (Koyama, E. et al (1996) *J Orthop Res.* 14(3):403-412 and Hall, BK. & Miyake, T. (1995) *Int J*

Dev Biol. 39(6):881-893) as well as in many diseases involving infections and inflammation (Schenk, S. et al. (1995) Int J Cancer 61(4):443-9).

To date there is only one report on tenascin-W available in the literature

5 (Weber, P. et al. (1998) J Neurobiol 35(1):1-16). In this study, a cDNA encoding tenascin-W was isolated from a 20-28h postfertilization zebrafish cDNA library on the basis of the conserved epidermal growth factor-like domains found in all tenascin molecules. The expression pattern of tenascin-W was studied in the developing zebrafish. It suggests an involvement of

10 tenascin-W in neural crest and sclerotome cell migration, as well as in the formation of the skeleton. Genebank sequence AJ001423 provides a zebrafish tenascin-W, and AL049689 provides a "novel human mRNA from chromosome 1, similar to Tenascin-R", whose function is not known.

15 The present invention provides a composition comprising an isolated nucleic acid molecule having a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence as set forth in SEQ ID NO: 1;

(b) a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 2;

20 (c) a nucleotide sequence with at least 85% identity to the sequence of (a) or (b);

(d) a subsequence of more than 50 consecutive nucleotides of a sequence of (a), (b) or (c); and

25 (e) a nucleotide sequence complementary to any of the nucleotide sequences or subsequence in (a),(b), (c), or (d).

In one aspect of the invention, the isolated nucleic acid molecule having a nucleotide sequence preferably exhibits at least 85% identity to the sequence of (a), more preferably encoding a variant of the amino acid sequence shown in SEQ ID NO: 2, such as a variant comprising an amino acid deletion, addition (e.g. fusion proteins) or substitution of the amino acid sequence

10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200 205 210 215 220 225 230 235 240 245 250 255 260 265 270 275 280 285 290 295 300 305 310 315 320 325 330 335 340 345 350 355 360 365 370 375 380 385 390 395 400 405 410 415 420 425 430 435 440 445 450 455 460 465 470 475 480 485 490 495 500 505 510 515 520 525 530 535 540 545 550 555 560 565 570 575 580 585 590 595 600 605 610 615 620 625 630 635 640 645 650 655 660 665 670 675 680 685 690 695 700 705 710 715 720 725 730 735 740 745 750 755 760 765 770 775 780 785 790 795 800 805 810 815 820 825 830 835 840 845 850 855 860 865 870 875 880 885 890 895 900 905 910 915 920 925 930 935 940 945 950 955 960 965 970 975 980 985 990 995 1000 1005 1010 1015 1020 1025 1030 1035 1040 1045 1050 1055 1060 1065 1070 1075 1080 1085 1090 1095 1100 1105 1110 1115 1120 1125 1130 1135 1140 1145 1150 1155 1160 1165 1170 1175 1180 1185 1190 1195 1200 1205 1210 1215 1220 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NO: 2, more preferably the variant has stem cell differentiation inducing activity, in particular an activity that induces osteoblast development from stem cells. Most preferred is when the isolated nucleic acid molecule encodes a protein with the amino acid sequence shown in SEQ ID NO: 2.

5

The nucleic acid molecule can be an antisense molecule, in which case it might be desirable to have nucleotide residues that are resistant to nuclease degradation substituting some or all of the ribo- or deoxyribonucleotides.

10 Also provided are nucleic acid vectors comprising the nucleic acid molecules of the invention, as well as host cells comprising the vectors or nucleic acids, and transgenic, knockout or genetically modified animals (other than humans, in particular mice), comprising manipulated nucleic acids of the invention or absent the endogenous sequence.

15

The invention also provides a composition comprising an isolated polypeptide having an amino acid sequence selected from the group consisting of:

- (a) an amino acid sequence as in SEQ ID No. 2; and
- (b) an amino acid sequence with at least 85% identity to the

20 sequence of (a); and

- (c) a subsequence of at least 30 consecutive amino acids of the sequence of (a) or (b), with the proviso that said subsequence does not fall within amino acid nos. 1102 and 1152 of SEQ ID NO:2.

25 Preferably, the amino acid sequence in (b) comprises a conservative substitution of at least one amino acid of the amino acid sequence of SEQ ID: NO: 2. More preferably, the polypeptide or fragment has stem cell differentiation inducing activity, as described above. Useful fragments may exhibit an epitope recognized by polyclonal antibodies raised against the

30 polypeptide having the amino acid sequence shown in SEQ ID NO: 2. for example. A particularly preferred polypeptide is that encoded by the amino acid sequence shown in SEQ ID NO: 2. Also provided are antibodies that are specifically reactive against the polypeptides of the invention.

In another aspect of the invention, a composition comprising an isolated nucleic acid molecule having a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence as set forth in SEQ ID No. 1 or SEQ ID No. 3;
- 5 (b) a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 2 or SEQ ID NO: 4;
- (c) a nucleotide sequence with at least 35% identity to any one of the sequences of (a) or (b), preferably (a);
- 10 (d) a subsequence of a least 15 consecutive nucleotides of the sequence of (a),(b) or (c); and
- (e) a nucleotide sequence complementary to (a),(b), (c), or (d), and a pharmaceutically acceptable excipient, diluent or carrier.

In one embodiment, the nucleic acid molecule preferably encodes a protein having stem cell differentiation inducing activity. In another embodiment, the nucleic acid molecule has a subsequence that is antisense to SEQ ID NO:1 or SEQ ID NO:3, wherein the nucleic acid molecule may comprise nucleotide residues that are resistant to nuclease degradation. In another embodiment, the isolated nucleic acid molecule encodes the amino acid sequence shown in SEQ ID NO: 2 or SEQ ID NO:4. In yet another embodiment, the nucleic acid molecule has a subsequence selected from the group consisting of nucleotides 2380-3171 of SEQ ID No:1, nucleotides 2371-3162 of SEQ ID No:3, a complement of nucleotides 2380-3171 of SEQ ID No:1, and a complement of nucleotides 2371-3162 of SEQ ID No:3, or an RNA equivalent thereof.

Thus, also provided are nucleic acid compositions as described above for use as a pharmaceutical, as well as for the manufacture of a medicament, in particular for the prophylaxis or treatment of cancer or bone pathologies.

30 Also provided are compositions comprising tenascin-W, preferably recombinant tenascin-W, and a pharmaceutically acceptable excipient, diluent

- (a) an amino acid sequence as set forth in SEQ ID No. 2 or 4;
- (b) an amino acid sequence with at least 35% identity to the sequence of (a); and
- (c) a subsequence of at least 30 consecutive amino acids of the sequence of (a) or (b).

5

Preferably, the polypeptide has stem cell differentiation inducing activity as described above. More preferably, the polypeptide is encoded by the amino acid sequence shown in SEQ ID NO: 4.

10

Thus, also provided is the use of tenascin-W for the treatment or prophylactic treatment of any disease or condition requiring increased tenascin-W levels, e.g. thrombosis, wound healing or atherosclerosis, as well as a condition ameliorated by the promotion of osteogenesis, e.g. bone healing, osteoporosis, as well as the use of tenascin-W as a stem cell marker.

15

Also provided are antibodies that specifically recognizes tenascin W for use as a pharmaceutical, as well as for the manufacture of a medicament, for the prophylaxis or treatment of cancer (e.g., glioblastoma, prostate, lung, colorectal, osteo- or breast carcinoma), including metastatic cancer, or for the prophylaxis or treatment of any disease or condition involving tenascin-W, e.g. excessive bone growth.

20

The present invention also provides methods for identifying agents for the prevention or the prophylactic treatment of tumourigenesis or the treatment or prophylactic treatment of tumours or cancer, or the treatment or prophylactic treatment of any disease or condition involving tenascin-W, e.g. a condition ameliorated by the promotion or inhibition of osteogenesis, comprising contacting a test compound with a tenascin-W expressing cell sample and then measuring a change in one or more of:

25

- (a) cell proliferation, e.g. cell progression entering S-phase of the cell cycle;
- (b) DNA synthesis;
- (c) cell adhesion;

30

- (d) cell spreading;
- (e) focal adhesion and actin stress fibre formation on fibronectin; and
- (f) cell binding to extracellular matrix (ECM)

relative to when said test compound is absent.

5

Optionally, the method further comprises measuring a change in tenascin-W expression relative to when the test compound is absent. The tenascin-W may have any one or more of the features described above. A particularly preferred assays is carried out in the form of an enzyme linked

10 immunosorbent assay (ELISA).

Thus also provided, are agents for the prevention or the prophylactic treatment of tumourigenesis or the diagnosis or the treatment or prophylactic treatment of tumours, or the treatment or prophylactic treatment of any disease or condition involving tenascin-W, e.g. a condition ameliorated by the promotion of osteogenesis, identified by a screening method of the invention.

Also provided are methods of diagnosing or prognosing cancer comprising:

- (a) obtaining a sample from an individual;
- (b) analysing said sample for the presence of tenascin-W; and
- (c) correlating the presence of tenascin-W with an unfavourable prognosis or diagnosis.

25 Optionally, the method may further comprise correlating in an increase in
tenascin-W in the sample relative to healthy tissue with an unfavourable
prognosis or diagnosis. The method may also include the additional use of
controls.

30 The present inventors have investigated extracellular matrix molecules, their expression during development, cell adhesion and proliferation of tumour cells and have characterized a novel member of the mammalian tenascin family.

Prior to the present invention, no lensarin-W had been identified from a

sequence encoding the mouse tenascin-W. Antisera were prepared against a fragment of tenascin-W, which detect tenascin W in tumour stroma, in the periosteum and in liver tissue, and cross react with tenascin W from several mammalian species. In particular, the inventors have discovered that

5 tenascin-W is specifically expressed in metastatic tumour cells as well as in the periosteum, the stem cell compartment for osteogenesis.

Thus, in one aspect, the present invention provides a composition comprising an isolated nucleic acid molecule having a nucleotide sequence selected from

10 the group consisting of:

- (a) a nucleotide sequence as set forth in SEQ ID NO: 1;
- (b) a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 2;
- (c) a nucleotide sequence with at least 85% identity to the sequence
- 15 of (a) or (b);
- (d) a subsequence of more than 50, 75, 100, 200 or more consecutive nucleotides of a sequence of (a), (b) or (c); and
- (e) a nucleotide sequence complementary to that of the nucleotide sequences or subsequence in (a), (b), (c), or (d).

20 The compositions include various types of nucleic acid, including genomic DNA, cDNA and mRNA, for example. In one aspect of the invention, the isolated nucleic acid molecule having a nucleotide sequence preferably exhibits at least 85% identity, more preferably 90% identity, most preferably 95, 98 or 100% identity to the sequence of (a). Also encompassed are nucleic acids that encode polypeptides having the amino acid sequence shown in

25 SEQ ID NO: 2, or variant thereof such as a variant comprising an amino acid deletion, addition (e.g. fusion proteins) or substitution relative to the amino acid sequence shown in SEQ ID NO:2. The various nucleic acids that can

30 encode these polypeptides therefore may differ because of the degeneracy of the genetic code, in that most amino acids are encoded by more than one triplet codon. The identity of such codons is well known in this art, and this information can be used for the construction of the nucleic acids within the scope of the invention. Variants differ from wild-type protein in having one or

more amino acid substitutions that either enhance, add, or diminish a biological activity of the wild-type protein. Once the amino acid change is selected, a nucleic acid encoding that variant is constructed according to methods well known in the art.

5 Preferably, the variant comprises a conservative substitution of at least one amino acid in said amino acid sequence in SEQ ID: NO: 2, more preferably the variant has stem cell differentiation inducing activity, in particular an activity that induces osteoblast development from stem cells. Most preferred is .

10 10 when the isolated nucleic acid molecule encodes a protein with the amino acid sequence shown in SEQ ID NO: 2.

The nucleic acid molecule can be an antisense molecule, in which case it might be desirable to have nucleotide residues that are resistant to nuclease 15 degradation substituting some or all of the ribo- or deoxyribonucleotides.

Also provided are nucleic acid vectors comprising the nucleic acid molecules of the invention, as well as cells comprising the vectors or nucleic acids, and transgenic, knockout or genetically modified animals (other than humans, 20 in particular mice), comprising manipulated nucleic acids of the invention or absent the endogenous sequence.

The invention also provides a composition comprising an isolated polypeptide having an amino acid sequence selected from the group consisting of:

25 (a) an amino acid sequence as set forth in SEQ ID NO: 2;

(b) an amino acid sequence with at least 85% identity, preferably 90, 95, 98 or 100% identity to the sequence of (a); and

(c) a subsequence of at least 30, 40, 50, 75, 100 or more consecutive amino acids of the sequence of (a) or (b), with the proviso that said 30 subsequence does not fall within amino acid nos. 1102 and 1152 of SEQ ID NO:2.

NO:2. More preferably, the polypeptide or fragment has stem cell differentiation inducing activity, as described above. Useful fragments may exhibit an epitope recognized by polyclonal antibodies raised against the polypeptide having the amino acid sequence shown in SEQ ID NO: 2, for 5 example. A particularly preferred polypeptide is that encoded by the amino acid sequence shown in SEQ ID NO: 2, derived from mouse tissue.

Therefore, also included within the invention are variants and derivatives of the polypeptide described by SEQ ID NO:2 or fragment thereof, whether 10 produced by recombinant means or synthetic means or isolated from naturally occurring sources. For example, peptides having modified amino acids/peptide linkages, and peptides containing non-naturally occurring amino acids and/or cyclic peptides, which may have improved properties such as 15 stability or activity are included. In addition the peptides of the invention may be in the form or a fusion with another protein, for example, tags for the target delivery or detection of the polypeptide (including fragments thereof).

A "variant" in terms of amino acid sequence defines an amino acid sequence that differs by one or more amino acids from another, usually related amino 20 acid sequence. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties (e.g. replacement of leucine with isoleucine). Less likely, a variant may have "non-conservative" changes, e.g. replacement of a glycine with a tryptophan. Similar minor variations may also include amino acid deletions or insertions 25 (i.e. additions), or both. Guidance in determining which and how many amino acid residues may be substituted, inserted or deleted without abolishing activity (e.g., anti.cancer activity, osteoblast promoting activity, antigenic activity) may be found using computer programs well known in the art. Variants of the polypeptides of the invention include all forms of mutant 30 variants, for example wherein at least one amino acid is deleted or substituted. Any changes involving substitution of amino acids are preferably neutral or conservative substitutions. Other variants include proteins or polypeptides comprising at least one additional amino acid in the sequence,

and/or further comprising an additional amino acid sequence or domain, such as fusion proteins, as is well known in the art.

Further variants of the polypeptides of the invention include those wherein at least one of the amino acids in the sequence is a natural or unnatural analogue. Also, one or more amino acids in the sequence may be chemically modified, e.g. to increase physical stability or to lower susceptibility to enzymic, particularly protease or kinase, activity.

10 Also provided are antibodies that are specifically reactive against the polypeptides of the invention. Methods for producing antibodies are well known in the art. An antibody specific for the polypeptide of the invention can be easily obtained by immunizing an animal with an immunogenic amount of the polypeptide. Therefore, an antibody recognizing the polypeptide of the invention embraces either of polyclonal antibody and antiserum which are obtained by immunizing an animal, and which can be confirmed to recognize the polypeptide of this invention by Western blotting, ELISA, immunostaining or other routine procedure known in the art.

15

20 It is well known that if a polyclonal antibody can be obtained by sensitization, a monoclonal antibody is secreted by the hybridoma, which may be obtained from the lymphocytes of the sensitized animal (Chapter 6, Antibodies A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1988). Therefore, monoclonal antibodies recognizing the polypeptide of the invention are also provided. Methods of producing polyclonal and monoclonal antibodies are known to those of skill in the art and described in the scientific and patent literature, see, e.g., Coligan, Current Protocols in Immunology, Wiley/Green, NY (1991); Stites (eds.) Basic and Clinical Immunology (7th ed.) Lange Medical Publications, Los Altos, CA, and references cited therein (Stites); 25 Goding, Monoclonal Antibodies: Principles and Practice (2nd ed.) Academic Press, New York, NY (1986); and Kohler (1975) *Nature* 256: 495. Such 30 techniques include selection of antibodies from libraries of recombinant

expressed by transient or stable expression vectors in mammalian cells, as in Norderhaug (1997) *J. Immunol. Methods* 204: 77-87.

In this invention, an antibody also embraces an active fragment thereof. An
5 active fragment means a fragment of an antibody having activity of antigen-
antibody reaction. Specifically named, these are active fragments, such as
F(ab')2, Fab', Fab, and Fv. For example, F(ab')2 results if the antibody of this
invention is digested with pepsin, and Fab results if digested with papain. Fab'
10 results if F(ab')2 is reduced with a reagent such as 2-mercaptoethanol and
alkylated with monoiodoacetic acid. Fv is a mono active fragment where the
variable region of heavy chain and the variable region of light chain are
connected with a linker. A chimeric antibody is obtained by conserving these
active fragments and substituting the fragments of another animal for the
fragments other than these active fragments. In particular, humanized
15 antibodies are envisioned.

The nucleic acid and polypeptide sequences indicated herein have been
found to be differentially expressed in samples obtained from metastatic
cancer cell lines and are predictive of tenascin-W expression in metastatic
20 cancer tissue, as well as in other types of cancer and diseases.

Accordingly, certain aspects of the present invention relate to nucleic acids
differentially expressed in tumour tissue, especially metastatic cancer cell
lines, polypeptides encoded by such nucleic acids, and antibodies
25 immunoreactive with these polypeptides, and preparations of such
compositions. Moreover, the present invention provides diagnostic and
therapeutic assays and reagents for detecting and treating disorders involving,
for example, aberrant expression of the subject nucleic acids.

30 Thus, in a further aspect of the invention, a composition is provided
comprising an isolated nucleic acid molecule encoding tenascin W or a
fragment thereof and a pharmaceutically acceptable excipient, diluent or
carrier. The pharmaceutical use of nucleic acids encoding tenascin W has not
previously been suggested and therefore in this embodiment, the nucleic

acids of the pharmaceutical compositions are not limited to the nucleic acids of the invention. In particular, the composition may comprise an isolated nucleic acid having a nucleotide sequence selected from the group consisting of:

- 5 (a) a nucleotide sequence as set forth in SEQ ID No. 1 or SEQ ID No. 3 (encoding human tenascin.W);
 (b) a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 2 or SEQ ID NO: 4;
 (c) a nucleotide sequence with at least 35% identity, preferably at least 10, 40, 50, 60, 70, 80, 90, 95 or 100% identity to any one of the sequences of (a) or (b), preferably (a);
 (d) a subsequence of at least 10, 15, 20, 25, 30, 40, 50, 75, 100 or more consecutive nucleotides of the sequence of (a),(b) or (c); and
 (e) a nucleotide sequence complementary to (a),(b), (c), or (d),
- 15 and a pharmaceutically acceptable excipient, diluent or carrier.

In one embodiment, the nucleic acid molecule encodes tenascin-W having the amino acid sequence as SEQ ID NO: 2 or SEQ ID NO. 4 or an amino acid with at least 30%, preferably at least 50%, 70%, 80%, 90%, 95%, or 100% identity to a sequence corresponding to SEQ ID NO:2 or 4. The nucleic acid molecules are at least 10, preferably at least 15, 20, 30, 50, 75, 100 or more consecutive nucleotides of SEQ ID No. 1 or SEQ ID No.2 or a sequence complementary thereto.

- 25 In one embodiment, the invention provides a composition comprising a nucleotide sequence fragment selected from the group consisting of nucleotides 2380-3171 of SEQ ID No:1 or nucleotides 2371-3162 of SEQ ID No:3, a complement of nucleotides 2380-3171 of SEQ ID No:1 or of nucleotides 2371-3162 of SEQ ID No:3, and RNA equivalents thereof, which encode an epitope for the binding with an antibody paratope.
- 30

In another embodiment, the nucleic acid molecule preferably encodes a sequence of human tenascin-W, differentiation, including a nucleic acid molecule

the isolated nucleic acid molecule preferably encodes the amino acid sequence shown in SEQ ID NO: 2 or SEQ ID NO:4, most preferably that of SEQ ID NO:4.

- 5 In yet another embodiment, the nucleic acid molecule has a subsequence that is antisense to SEQ ID NO:1 or SEQ ID NO:3, wherein the nucleic acid molecule may comprise nucleotide residues that are resistant to nuclease degradation.
- 10 The nucleic acid may be antisense to all or a part of a nucleic acid which hybridizes under stringent conditions to SEQ ID No:1 or SEQ ID No:3, or antisense to a sequence having at least 70% identity with SEQ ID NO:1 or SEQ ID NO:3, that is able to hybridize under low stringency conditions to SEQ ID NO:1 or SEQ ID NO:3, and which encodes tenascin-W. Low stringency
- 15 conditions employs around 0.01 x SSC buffer compared to high stringency which employs about a 10 fold greater concentration. Alternatively, the antisense RNA may be antisense to regulatory sequences of the tenascin-W gene, in particular to 5' upstream sequences (promoter region) of the gene. Similarly, small RNAi oligonucleoties can be designed to inhibit expression of
- 20 Tenascin-W in a specific manner.

The nucleic acids can be RNA or DNA, sense or antisense, and in some embodiments, double stranded or single stranded. In certain embodiments at least some of the nucleotide residues of the nucleic acids (sense or antisense)

- 25 may be made resistant to nuclease degradation and these can be selected from residues such as phosphorothioates and/or methylphosphonates.

The antisense nucleic acids as hereinbefore described can advantageously be used as pharmaceuticals, preferred pharmaceutical applications being for

- 30 the manufacture of a medicament for the prophylaxis or treatment of conditions dependent on elevated Tenascin W levels, such as cancer.

Thus, the invention also provides a method of preventing or treating a condition dependent on Tenascin W, comprising administering to an individual

an effective amount of a nucleic acid, as hereinbefore described. Thus, the invention encompasses the use of such nucleic acid molecules as a pharmaceutical, as well as for the manufacture of a medicament, in particular for the prophylaxis or treatment of cancer or bone pathologies.

5

In yet another aspect, the present invention provides expression vectors capable of replicating in a host cell, comprising one or more vector sequences and a nucleic acid sequence encoding teneurin-W. The construct for use as a pharmaceutical is also provided, as well as its use for the manufacture of a 10 medicament for the prophylaxis or treatment of cancer or the prophylaxis or treatment of bone pathologies.

Other embodiments of the invention include nucleic acid constructs capable of replicating in a host cell, comprising (a) at least one nucleic acid sequence 15 portion encoding a tenascin-W protein or polypeptide of the invention (b) antisense nucleic acids as hereinbefore described (or their complement, for example, if expression of the antisense RNA in a cell is foreseen), or (c) nucleic acids as hereinbefore described and at least one nucleic acid sequence encoding a protein other than tenascin-W (or its homologues), e.g 20 vector sequence. Such constructs are not naturally occurring sequences.

The constructs lack essential sequences of DNA which might permit them to function as vectors but are not naturally occurring as "hybrid" nucleic acids. They may include nucleic acid sequences that function as linkers or restriction sites which include without limitation a transcriptional regulatory sequence 25 operably linked to a nucleotide sequence of the invention so as to render said nucleic acid construct capable of replicating in a host cell. Preferred constructs are synthesised using methods of oligonucleotides synthesis well known to those of skill in the art.

30 Also provided are vectors comprising a construct as hereinbefore described. Preferred vectors are expression vectors, preferably plasmids or viruses. Alternatively, viral vectors are also provided for, optionally in the form of

The invention provides host cells containing vectors. Preferred host cells are eukaryotic cells, more preferably insect cells or mammalian cells.

Constructs, vectors and transformed host cells of the invention are of use as 5 pharmaceuticals, as well as for the manufacture of a medicament for the prophylaxis or treatment of a condition dependent on tenascin W.

Similarly, in a further aspect of the invention, a composition is provided comprising tenascin W, preferably recombinant tenascin-W, or a fragment 10 thereof and a pharmaceutically acceptable excipient, diluent or carrier. In preferred embodiments, the tenascin-W is a polypeptide having an amino acid sequence selected from the group consisting of:

- (a) an amino acid sequence as set forth in SEQ ID No. 2 or 4;
- (b) an amino acid sequence with at least 35% identity, preferably at least 15 50%, 70%, 80%, 90%, 95%, or 99% identity to the sequence of (a); and
- (c) a subsequence of at least 5, 10, 15, 20, 30, 50, 70, 100 or more consecutive amino acids of the sequence of (a).

Preferably, the polypeptide has stem cell differentiation inducing activity as 20 described above. More preferably, the polypeptide is encoded by the amino acid sequence shown in SEQ ID NO: 4.

Thus, also provided is the use of tenascin-W for the treatment or prophylactic treatment of any disease or condition requiring increased tenascin-W levels, 25 e.g. thrombosis, wound healing or atherosclerosis, as well as a condition ameliorated by the promotion of osteogenesis, e.g. bone healing, osteoporosis, as well as the use of tenascin-W as a stem cell marker.

In yet a further aspect, the tenascin-W protein is used as a pharmaceutical.

30 The present invention further provides the use of a tenascin-W, e.g. for the manufacture of a medicament, for the prevention or prophylactic treatment of tumourigenesis or the treatment or prophylactic treatment of tumours or cancer. The invention also includes the use of the tenascin-W for the manufacture of a medicament for the treatment or prophylactic treatment of

any one or more of bone disease, rheumatism, asthma, allergic diseases, autoimmune diseases, prevention of transplant rejection and any other diseases involving tenascin e.g. thrombosis, cancer, wound healing and atherosclerosis.

5

The invention therefore provides pharmaceutical compositions for humans or veterinary compositions for animal use that comprise one or more of the aforementioned active fragments of tenascin-W. The compositions may also include other active or non-active agents. Non-active agents may include a 10 pharmaceutically acceptable excipient, diluent or carrier, but not limited to saline, buffered saline, dextrose and water.

The compositions and medicaments of the invention may therefore be used prophylactically in order to prevent tumours from forming, or they may be used 15 in a curative or partly curative way to treat or contain a pre-existing tumourous condition. As well as tumours ~~and serious~~ of malignant conditions may be prevented or treated with compositions or medicaments of the invention.

In a particular aspect, the present invention provides the use of the nucleic 20 acid or proteins or polypeptides as hereinabove described, for radioimmunotherapy. The same therapy has been described for brain tumours with overexpression of tenascin-C. Use of radiolabeled antibody is a promising approach to target radiotherapy directly into the tumor. Anti-tenascin-C antibodies are currently tested in phase I and II clinical trials. 25 There are many reports in the literature on the progress of these clinical trials. Patients with malignant gliomas were administrated locoregional radioimmunotherapy (LR-RIT) using ¹³¹I-labeled anti-tenascin antibody injected directly in the tumor (Riva et al., 1999a). The first results show that LR-RIT can be safely performed, with good results especially in patients with 30 minimal disease. Similar approach was performed with ⁹⁰Y (a pure beta emitter)-labeled antibodies Riva et al., 1999b), with promising results.

Potentially more efficient radicimmunotherapies were shown to be possible using other approaches like the use of ¹³¹I-labeled anti-tenascin antibody

is now initiated in a phase I clinical study. It is as well a useful tool for precise imaging of tumors, since the presence of isotopes specifically targeted into the tumor allows sequential scintigraphies of the tumor (Riva et al., 1999a), and makes possible a direct estimation of the success of the therapy.

5

The tumours or tumour cells of the present invention are preferably those which express tenascin-W in the stroma. In particularly preferred embodiments the tumours are solid tumours, e.g. mesenchymal tumours such as osteosarcoma, glioblastoma or epithelial cancers such as breast, prostate,

10 lung and colorectal carcinoma.

The invention further provides the use of tenascin-W for the treatment or prophylactic treatment of a condition ameliorated by the promotion of osteogenesis, e.g osteoporosis, osteoarthritis, treatment of cartilage and bone

15 pathologies. A protein or polypeptide as hereinabove described may be used to be incorporated into implants including without limitation hip joints, knee joints, or broken bones, to promote osteogenesis.

The invention also provides a method of preventing or prophylactic treatment 20 of tumourigenesis or of treatment or prophylactic treatment of tumours or cancer or of any one or more of rheumatism, asthma, allergic diseases, autoimmune diseases, prevention of transplant rejection or the treatment or prophylactic treatment of any disease involving tenascin-W, e.g., thrombosis, wound healing and atherosclerosis in an individual comprising administering 25 an effective amount of a tenascin-W or a fragment thereof.

The invention also provides a method of treatment or prophylactic treatment of 30 a condition ameliorated by the promotion of osteogenesis, e.g osteoporosis, osteoarthritis, treatment of cartilage and bone pathologies in an individual comprising administering an effective amount of tenascin-W or a fragment thereof.

The determination of an effective dose is well within the capability of those skilled in the art. For any compound, the therapeutically effective dose can be

estimated initially either in cell culture assays or in an appropriate animal model. The animal model is also used to achieve a desirable concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

5 A therapeutically effective dose refers to that amount of active agent which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals (e.g., ED₅₀, the dose therapeutically effective in 50% of the population; and LD₅₀, the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD₅₀/ED₅₀.

10 Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

15

20 The exact dosage may be chosen by the individual physician in view of the patient to be treated. Dosage and administration can be adjusted to provide sufficient levels of the active moiety or to maintain the desired effect.

Additional factors which may be taken into account include the severity of the disease state (e.g. tumour size and location); age, weight and gender of the patient; diet; time and frequency of administration; drug combination(s); reaction sensitivities; and tolerance/response to therapy. Long acting pharmaceutical compositions can be administered on a daily basis, every 3 to 4 days, every week, or once every two weeks, depending on half-life and

25

30 clearance rate of the particular formulation.

The present inventors have observed stem cells, especially the perineurium,

which are believed to be a source of stem cells for the formation of the

present compositions. It is believed that the stem cells are present in the

wherein tenascin-W is used as a stem cell marker for cells including without limitation osteogenic precursor cells in the bone marrow. Therefore, also provided is a method of selecting stem cells or progenitor cells having the ability to differentiate into osteoblasts from other cells, not having this ability.

- 5 Stem cells expressing tenascin-W can be detected by an antibody. The antibody recognizing tenascin-W can be detected using secondary antibodies specific for the tenascin-W antibody, which are optionally labelled with a radiolabel, an enzyme, avidin or biotin, or fluorescent materials (e.g. green fluorescent protein (GFP) or rhodamine), for example. The cells are
- 10 characterized by having tenascin-W expression above basal levels and are preferably selected from a mixed population of cells using the fluorescence-activated cell sorter (FACS) (see for example Abe et al., Dev Biol. 1996; 180(2):468-72). The selected cells therefore carry a protein detectable by fluorescence. The sorted cells are useful for the production of biological parts
- 15 of the body, e.g. bone tissue.

Also provided are antibodies that specifically recognize tenascin-W for use as a pharmaceutical, as well as for the manufacture of a medicament for the prophylaxis or treatment of cancer (e.g., glioblastoma, prostate, lung, colorectal, osteo- or breast carcinoma), including metastatic cancer, or for the prophylaxis or treatment of any disease or condition involving tenascin-W, e.g. excessive bone growth. In another aspect of the present invention, an antibody specifically reactive against tenascin-W or a fragment thereof, and the use of an antibody for the manufacture of a medicament for the prophylaxis or treatment of cancer, and the antibody for use as pharmaceutical is provided.

Antibodies that specifically recognize tenascin-W or a fragment thereof are also provided, in particular antibodies that recognise the above mentioned epitope.

Methods for detecting tenascin-W embrace, for example, the use of an antibody as referred to above, optionally with the use of an enzyme reaction. The antibody recognizing tenascin-W can be detected using secondary

antibodies specific for the tenascin-W antibody, which are optionally labelled with a radiolabel, an enzyme, avidin or biotin, or fluorescent materials (FITC or rhodamine), for example.

- 5 Also encompassed by the invention is the use of an antibody that specifically recognizes tenascin-W for the manufacture of a medicament, in particular a medicament for the prophylaxis or treatment of cancer, the prophylaxis or treatment of bone disease, or as a pharmaceutical. In particularly encompassed by the invention is the use of an antibody that specifically
- 10 recognizes tenascin-W for the diagnosis of tumour, especially metastatic tumour.

In a further embodiment, the present invention provides a method for identifying agents for the prevention or the prophylactic treatment of

- 15 tumourigenesis or the treatment or prophylactic treatment of tumours or cancer, or the treatment or prophylactic treatment of any disease or condition involving tenascin-W, e.g. a condition ameliorated by the promotion (or inhibition) of osteogenesis, comprising combining a test compound with a tenascin-W expressing sample and then measuring a change in one or more
- 20 of (a) cell proliferation, e.g. cell progression entering S-phase of the cell cycle; (b) DNA synthesis; (c) cell adhesion; (d) cell spreading; (e) focal adhesion and actin stress fibre formation on fibronectin; (f) cell binding to extracellular matrix (ECM), relative to when said test compound is absent.

25 Cells may be encouraged to proliferate by the addition of tenascin-W to the cell culture, preferably by coating the solid substrate therewith. A substrate can be any surface that promotes cell adhesion. The solid substrate may also be coated by other ECM which include without limitation fibronectin, collagen, etc. The cell cultures are preferably grown on a solid substrate or in a liquid

- 30 medium. A first measurement of one or more of (a) to (f) may be made prior to contacting the cells with a test substance. A second measurement may be made thereafter. A multiplicity of further measurements may be made over a

this way a time course of the cellular response(s) may be obtained and analysed.

In one preferred embodiment of the present invention, the presence of 5 tenascin-W in the liquid medium is measured relative to when a test compound is absent. An increase in the level of tenascin-W present in the medium relative to when said test agent is absent correlates to an agent effective in the promotion of osteogenesis. A decrease in the level of tenascin-W present in the medium relative to when said test agent is absent correlates to an anti-proliferative or anti-tumour agent, or an agent effective in inhibiting 10 osteogenesis or osteoblast formation.

In preferred aspects one or more of the following conditions arising after 15 contacting cells with a test compound is indicative of an anti-proliferative or anti-tumour agent, or an inhibitor of osteoblast formation:

- (a) a reduction in cell proliferation; or a decrease in the proportion of 20 cells entering S-phase of the cell cycle;
- (b) a reduction in DNA synthesis;
- (c) an increase in cell adhesion;
- (d) an increase in cell spreading;
- (e) an increase in focal adhesion and actin stress fibre formation on fibronectin; and
- (f) an increase in the binding of cells to ECM, preferably fibronectin;

25

In other preferred aspects one or more of the following conditions arising after 30 contacting cells with a test compound may indicate an osteogenesis promoting agent:

- (a) an increase in cell proliferation; or an increase in the proportion of cells entering S-phase of the cell cycle;
- (b) an increase in DNA synthesis;

(c) and (d) an increase in the expression of bone-specific markers such as alkaline phosphatase activity, calcification or any others known in the art (e.g., Raouf and Seth, 2002, *Bone* 30: 463-71).

5 Actin stress fibre formation may be assayed according to the Actin Assembly Assay described in Bloom, L *et al* (1999) *Mol Biol Cell* **10**: 1521-1536. Adhesion assays may be performed according to the method described in Bloom, L *et al* (1999).

10 In other embodiments, the method of the invention may further comprise control cells grown in the absence of test substance and (a), (b), (c), (d), (e), and/or (f) are measured in both control and test cultures. The test measurements can thereby be normalised with respect to the control.

15 The screening method further provides an essentially cell-free system for the identification of potential anti-tumour or tumour preventing agents or for an agent inhibiting osteogenesis. This method relies on the ability of a potential anti-tumour agent to prevent, inhibit or disrupt the binding between an ECM and tenascin-W. The nature of any disruption of the ECM and tenascin-W

20 binding may be determined by performing a binding assay for ECM and tenascin (see e.g. example 9). For example, calorimetric methods may be used or measurement of labelled reagents. The relative amounts or concentrations of reagents and test substance may be varied, thereby permitting calculation of inhibition constants and other parameters, e.g.

25 binding affinities. The optimisation of assay conditions will be well within the realm of one of ordinary skill in the art.

The system may further comprise a control without test substance and the binding is measured in the control, thereby permitting corresponding

30 measurements in the test system to be normalised relative to the control.

Where one component of the assay (screening) systems of the invention is

radiolabels e.g. ^{14}C or ^3H , dyes, metal sols, enzymes or biotin/avidin. By attaching such labels to "free" components in the system any binding assay may be carried out in solution in accordance with procedures well known in the art. After allowing the components to react solid phase particles can be

5 separated from solution, e.g. by filtration or sedimentation (including centrifugation). In some embodiments immunoprecipitation may be used to separate bound and free labelled components. Usually, an antibody may be employed to bring an unlabelled component out of solution (whether or not this component has bound to another labelled component or not). After

10 separation, the label present in solution (free) and the label present in or on the solid phase (bound) may be measured. Standard analyses of such bound and free data, e.g. Scatchard plots and the determination of affinity and inhibition constants for binding are well known to the person of ordinary skill in the art.

15 Where the solid phase is not particulate, e.g. in the form of a surface, such as a microtiter plate well, then binding assays measuring bound and free label may be performed but this will normally involve the removal of reaction components from the wells after binding reactions have occurred. Advantageously, this

20 assay format may dispense with the need for providing specifically labelled reaction components. Instead, labelled antibodies may be used to measure the binding of previously free reaction components to solid phase components.

25 In some embodiments the tenascin-W molecule, variant or fragment thereof may be attached directly to a solid phase. In preferred immunoassay embodiments of this type, tenascin-W bound to an ECM is measured using an antibody reactive against tenascin-W.

30 Immunological binding assays are known in the art. For a review, see Methods in Cell Biology Vol. 37: Antibodies in Cell Biology, Asai, (Ed.) Academic Press, Inc. New York (1993).

A label may be any detectable composition whereby the detection can be spectroscopic, photochemical, biochemical, immunochemical, physical or chemical. For example, useful labels can include ^{32}P , ^{35}S , ^3H , ^{14}C , ^{125}I , ^{131}I , fluorescent dyes (e.g. FITC, rhodamine and lanthanide phosphors), electron-dense reagents, enzymes, e.g. as commonly used in ELISA (e.g. horseradish peroxidase, beta-galactosidase, luciferase and alkaline phosphatase), biotin, dioxigenin, or haptens and proteins for which antisera or monoclonal antibodies are available. The label may be directly incorporated into a target compound to be detected, or it may be attached to a probe or antibody which binds to the target.

Throughout the assays of the invention, incubation and/or washing steps may be required after each application of reagent or incubation of combinations of reagents. Incubation steps may vary from about 5 minutes to several hours, perhaps from about 30 minutes to about 6 hours. However, the incubation time usually depends upon the assay format, analytic volume of solution, concentrations, and so forth. Usually, the assays should be carried out at ambient temperature, although they may be conducted at temperatures; in the range 10°C to 40°C, for example.

A particularly preferred assay format is an enzyme-linked immunosorbent assay (ELISA).

All of the aforementioned methods of screening of the invention are equally applicable to the screening of substances for biological activity and potential agents for any other disease or condition involving tenascin-W, e.g. wound healing or treatment of atherosclerosis.

Also included within the scope of the present invention are anti-tumourigenic, anti-tumour, anti-metastatic, (anti-)osteogenic, wound healing or anti-atherosclerosis substances or substances for the treatment or prophylactic treatment of any disease or condition involving tenascin-W identified by any of

includes pharmaceutical compositions for preventing or treating tumours, metastasis, or bone pathologies and comprising one or more of the substances identified by a method of the invention. For example, inhibitors of tenascin-W expression or activity are considered potential anti-cancer agents, 5 whereas tenascin W or agonists thereof are considered agents effective in promoting osteogenesis, which can be used in vivo or ex vivo.

Thus, the present invention provides a novel mammalian member of the tenascin family and uses thereof. It permits the identification of agents 10 effective against conditions dependent on tenascin-W, in particular anti-cancer agents or agents that promote osteogenesis, by performance of any of the methods of screening described herein. Preferred anti-cancer agents are those which inhibit proliferation of the cancer cells and which may be general anti-proliferative agents.

15 The invention includes all nucleic acid molecules and proteins and polypeptides, as hereinabove described, as well as agents identified by performing the experiments, and the use of these agents as pharmaceuticals, particularly as medicaments for the prophylaxis or treatment of cancer and 20 other conditions dependent on tenascin W.

Thus, in a further aspect the invention provides for the use of tenascin-W and of an agent identified by a screening method of the invention as a pharmaceutical.

25 The invention further provides tenascin-W or an agent identified by a screening method of the invention, for the manufacture of a medicament for the prophylaxis or treatment of a condition dependent on tenascin-W, for use to treat cancer or bone diseases or an immunological defect.

30 The invention provides a method of preventing or treating a condition dependent on tenascin-W comprising administering to an individual an effective amount of a construct, vector, host cell or antibody described above.

The invention also provides a method of inhibiting a condition dependent on tenascin-W comprising administering an effective amount of the modulator identified by a screening method of the invention described above, for the treatment of cancer or bone disease or an immunological defect.

5

Also provided by the invention are the nucleic acid molecules, the proteins, and the agents referred to above in a pharmaceutical composition, possibly in the presence of suitable excipients known to one of ordinary skill in the art.

The compositions may be administered in the form of any suitable

10 composition as detailed below by any suitable method of administration within the knowledge of a skilled man. The preferred route of administration is parenterally. In parenteral administration, the compositions of this invention will be formulated in a unit dosage injectable form such as a solution, suspension or emulsion, in association with a pharmaceutically acceptable 15 excipient. Such excipients are inherently nontoxic and nontherapeutic.

Examples of such excipients are saline, Ringer's solution, dextrose solution and Hank's solution. Nonaqueous excipients such as fixed oils and ethyl 20 alcohol may also be used. A preferred excipient is 5% dextrose in saline. The excipient may contain minor amounts of additives such as substances that enhance isotonicity and chemical stability, including buffers and preservatives.

Any protein is administered at a concentration that is therapeutically effective to prevent allograft rejection, GVHD, allergy and autoimmune diseases. The dosage and mode of administration will depend on the individual. Generally, 25 the compositions are administered so that the functional protein is given at a dose between 1 pg/kg and 10 mg/kg, more preferably between 10 ug/kg and 5 mg/kg, most preferably between 0.1 and 2 mg/kg. Preferably, it is given as a bolus dose. Continuous short time infusion (during 30 minutes) may also be used. The compositions according to the invention may be infused at a dose 30 between 5 and 20 μ g/kg/minute, more preferably between 7 and 15 μ g/kg/minute.

According to a specific case, the "therapeutically effective amount" of a composition needed should be determined as being the amount sufficient to cure the patient in need of treatment or at least to partially arrest the disease and its complications. Amounts effective for such use will depend on the

5 severity of the disease and the general state of the patient's health. Single or multiple administrations may be required depending on the dosage and frequency as required and tolerated by the patient.

The present invention also provides a method of diagnosing or prognosing

10 cancer, or any other condition dependent on elevated tenascin W levels, comprising, (a) obtaining a sample from an individual; (b) analysing said sample for the presence of tenascin-W; and (c) correlating the presence of tenascin-W with a favourable prognosis or diagnosis.

15 The methods of the present invention will typically involve the determination of the presence, level, or activity of tenascin-W in a cell or tissue sample, which sample will often be obtained from a human, but one can also readily understand that samples tested by the present method can be obtained from agriculturally important mammals, such as cattle, horses, sheep, etc.

20 animals of veterinary interest, such as cats and dogs. The assay may be carried out on any cell or tissue sample, such as somatic tissues, germline tissues, or cancerous tissues, as well as on samples from body fluids, such as pleural fluid, blood, serum, plasma and urine.

25 A "sample" is the material being analyzed which is usually, but not necessarily, subjected to pretreatment to provide the tenascin-W in assayable form. This would normally entail forming a cell extract, methods for which are known in the art (for example, see Scopes, Protein Purification: Principles and Practice, Second Edition (Springer-Verlag, N.Y., 1987)).

30 In the broader aspects of the invention, there is no limitation on the collection and handling of samples as long as consistency is maintained. The sample is obtained by methods known in the art, such as, biopsies, surgical resections,

smears, or the like. Optionally, cells obtained in a sample may be propagated in cell culture.

Consistency of measurement of tenascin-W or tenascin-W activity in clinical samples can be ensured by using a variety of techniques. For example, to control for the quality of each tissue extract, another enzymatic activity, such as alkaline phosphatase, can serve as an internal control. In addition, an internal standard can be measured concurrently with tenascin-W in the sample as a control for assay conditions. Thus, the analyzing step can comprise detecting a control protein in the sample, optionally normalizing the value obtained for tenascin-W with a signal obtained with the control protein.

The presence of tenascin-W in the sample can be determined by detecting the tenascin-W protein using methods known in the art. In this invention, there are no limitations on the type of assay used to measure tenascin-W or tenascin-W activity. For example, tenascin-W can be detected by immunoassays using antibodies specific for tenascin-W. The antibody can be used, for example, in Western blots of two dimensional gels where the protein is identified by enzyme linked immunoassay or in dot blot (ELISA sandwich) assays of total cellular protein, or partially purified protein.

Methods for sample concentration and protein purification are described in the literature (see Scopes, 1987). For example, if desired, the tenascin-W present in the cell extract can be concentrated, by precipitating with ammonium sulfate or by passing the extract through a commercially available protein concentration filter, e.g., an Amicon or Millipore, ultrafiltration unit. The extract can be applied to a suitable purification matrix, such as an anion or a cation exchange resin, or a gel filtration matrix, or subjected to preparative gel electrophoresis. In such cases, the tenascin-W and protein yield after each purification step needs to be considered in determining the amount of tenascin-W in a sample.

Tenascin-W may be detected using an antibody specific for tenascin-W, and a test for cellular tenascin-W can be carried out using the methods described above.

tenascin molecule. Optionally, the method may further comprise correlating in an increase in tenascin-W in the sample relative to healthy tissue. For example, tenascin-W can be detected using an antibody specific for tenascin-W expressed in tumour tissue and compared to antibody binding to any

5 tenascin-W expressed (or non-specific reaction) in healthy tissue.

The sample is preferably a tissue sample mounted onto a solid surface for histochemical analysis. The presence of detectable, accessible tenascin-W indicates that tenascin-W is accessible to cells for binding. This leads to a

10 unfavourable diagnosis or prognosis. If, on the other hand, the antibody does not react with tenascin-W in the tissue section, then there is an expectation that tenascin-W is not present. This leads to a favourable diagnosis or prognosis.

15 The present inventors have found that tenascin-W is specifically expressed in solid tumours, in particular metastatic tumour tissue or stroma thereof. The presence of tenascin-W therefore indicates a cancerous condition, in particular the presence of metastatic tumour tissue, whereas the absence of tenascin-W indicates healthy tissue or non-metastatic tumour tissue. Two variants of 170 and 190 kD tenascin-W were identified in developing mouse tissues by western blotting. High expression of the large variant of tenascin-W was found in the metastatic tumours of ras-transgenic mice, but not in the myc- or neuT-transformed non-metastatic tumours. The large variant of tenascin-W (190 kD) is indicative of unfavourable diagnosis; whereas the 25 170kD variant is indicative of more favourable prognosis or healthy individual.

In a preferred embodiment, the invention provides kits suitable for use in the diagnostic or prognostic methods of the invention. Such kits comprise reagents useful for carrying out these methods, for example, antibodies from 30 one or more species specific for tenascin-W. Secondary antibodies that recognise either or both such primary anti-fibronectin antibodies can also be included for the purpose of recognition and detection of primary antibody binding to a sample. Such secondary antibodies can be labelled for detection e.g. with fluorophores, enzymes, radioactive labels or otherwise. Other

detection labels will occur to those skilled in the art. Alternatively, the primary anti-tenascin-W antibodies can be labelled for direction detection.

The invention is further described below, for the purpose of illustration only, in
5 the following examples.

Example 1: Cloning of mouse tenascin-W

Mouse tenascin-W was cloned from a cDNA library of 19d whole mouse embryos (DupLEX-A DLM-110; OriGene Inc.). In a first step the following PCR primers derived from a sequence from chromosome 1, similar to Tenascin (Accession number AL049689) were used for nested PCR reactions with the Expand High Fidelity PCR System (Roche) using the mouse cDNA library as template. The first reaction was performed with the primer set 5'-TAGCAGCCCCACAGCATCTACTTGCC-3' (SEQ ID NO:5) / 5'-ATTGCTGTTCTGCTAACCTGACTGCA -3' (SEQ ID NO:6) and the second reaction with 5'-ATGGATCCAGAAATTGACGGCCCCAAAAACCTAG-3' (SEQ ID NO:7) / 5'-ATAAGCTTGTGGAGAGGGTGGATACATTC-3' (SEQ ID NO:8). The second primer set included a BamHI and a HindIII restriction site, respectively, to enable the directed cloning into the bacterial expression vector pQE30 (Qiagen) supplying a C-terminal His-tag for the purification of the recombinant protein. The protein was expressed and purified by affinity chromatography to a Ni-NTA matrix (Qiagen) according to the manual of the supplier. The protein was purified under native conditions and was eluted with 250mM imidazole. The recombinant protein encompasses the three most C-terminal fibronectin type III repeats of tenascin-W (aa 794-1057 of the complete amino acid sequence of mouse tenascin-W encoded by nucleotides 2380 – 3171 of the tenascin-W nucleotide sequence). Full length tenascin-W was cloned by the use of mouse tenascin-W specific primers derived from the above mouse tenascin-W cDNA and primers matching the vector of the same 19d whole mouse embryo cDNA library used before. To complete the 5' part the following PCR reactions using the above cDNA as template were performed: The first PCR reaction was performed using the primer pair 5'-AGGAGATGGTGGTGTATTTCTGG-3' (SEQ ID NO:9) / 5'-GGTGGTGTATTTCTGG-3' (SEQ ID NO:10).

by a second PCR reaction with the primer set 5'-

TAGAATTCGGTACACCTGATTGGTCAGTAGG-3' (SEQ ID NO:11) / 5'-

TTATGATGTGCCAGATTATGCC-3' (SEQ ID NO:12). To complete the 3' part of the tenascin-W cDNA the following PCR reactions were performed:

5 In the first reaction the primer pair 5'-CTCAAATTGATGGCTACATTTGACC-3' (SEQ ID NO:13) / 5'-AAGCCGACAACCTTGATTGGAGAC-3' (SEQ ID NO:14) was used followed by the primer pair 5'-TACCAGTTCCAAATGGCACCG-3' (SEQ ID NO:15) / 5'-AAACCTCTGGCGAAGAAGTCC-3' (SEQ ID NO:16). In each case the longest products were cloned. These overlapping tenascin-W
10 cDNA clones were assembled into one full length mouse tenascin-W cDNA and cloned into the expression vector pCEP/Pu (see Kohfeldt et al. (1997). FEBS Lett. 414:557-61). At the 3'end of the tenascin-W cDNA a 6xHis-tag was inserted in front of the stop codon to allow the purification of full length mouse tenascin-W protein expressed in mammalian cell culture.

15

Example 2: Characterization of mouse Tenascin-W

The full length cDNA of mouse tenascin-W was cloned as described in example 1. The full length tenascin-W cDNA was transfected into HEK293 cells using the transfection reagent fugene (Roche). Transfected cells
20 selected for with puromycin and the medium containing the secreted tenascin-W protein was collected and the protein was purified by sequential chromatography over a gelatin-agarose column (Sigma) to remove any contaminating fibronectin in the preparation and by adsorption to a Ni-NTA matrix (Qiagen). The tenascin-W was eluted from the nickel column by
25 250mM imidazole. The cDNA sequence encodes a typical member of the tenascin protein family and harbors from the N-terminus to the C-terminus of the protein the following structural domains: signal peptide for secretion, N-terminal domain for dimerisation of two tenascin-W trimers that are assembled by the heptad repeats. This results in a disulfide-linked hexameric protein
30 complex where each subunit contains three and a half EGF-like repeats, nine fibronectin type III repeats, and a fibrinogen-like C-terminal globular domain. Furthermore, electronmicrographs of tenascin-W after rotary shadowing revealed hexameric molecules with the six subunits of 50nm length radiating from a central globular domain. The recombinant protein was analyzed by

SDS-PAGE (sodium dodecyl sulfate - polyacrylamide gel electrophoresis) on 6% polyacrylamide gels, under reducing and non-reducing conditions as well as by electron microscopy after rotary shadowing using the same procedure as described for tenascin-C (Chiquet-Ehrismann, R. et al. (1988) *Cell* **53**, 383 – 390). Tenascin-W showed a similarly slow migration as the hexameric tenascin-C protein. Two tenascin-W variants of 170 and 190 kD were identified in developing mouse tissues by western blotting. High expression of the large variant of tenascin-W was found in the metastatic tumors of ras-transgenic mice, but not in the myc- or neuT-transformed non-metastatic tumors. Expression of low amounts of the small variant of tenascin-W was found in healthy tissue, e.g. blood serum.

Example 3: Antibody production, immunohistochemistry and immunoblots

The bacterially expressed recombinant fragment of tenascin-W as described above in example 1, was used to raise polyclonal antisera in rabbits using standard immunization procedures as hereinabove ~~described~~. This antiserum reacted specifically with purified full length recombinant tenascin-W as well as with endogenous tenascin-W in tissue extracts of various tissues. These antisera were used to detect tenascin-W in tissue extracts and cryosections of developing mouse embryos as well as in mouse mammary tumours using the same methods as described for tenascin-Y (Hagios, C. et al. (1996) *J. Cell Biol.* **134**, 1499-1512). Briefly, tissues were fixed in ice-cold 4% paraformaldehyde in phosphate-buffered saline (PBS) overnight, washed with PBS and cryoprotected with 25% sucrose in PBS overnight at 4°C. The tissues were embedded in OCT (Optimal Cutting Temperature) mounting medium (Cat. No. 27050 OCT Compound by Ted Pella Inc., CA) and sections of 12-16 μ m were cut and collected onto glass slides. The sections were air-dried for 2 hours before staining with the anti-tenascin-W antiserum followed by a fluorescently labelled secondary antibody.

Example 4: Expression of tenascin-W during development

The anti-tenascin-W antiserum, as described in example 3, was used to investigate its expression during normal mouse development by immunohistochemistry. Tenascin-W first appears at embryonic day 11.5

5 (E11.5) in the maxillary process. Between E14.5 and E16.5 tenascin-W and tenascin-C overlap in developing connective tissue (palate and mandible) in the face and jaw. Furthermore tenascin-W is found in the ECM of smooth muscle, mesothelia and bone. In the adult mouse tenascin-W is found in a subset of the tenascin-C-positive ECM of the aortic valve and the limbus. In
10 these locations its expression coincides with the stem cell compartment of the respective tissue. Tenascin-W is also expressed in the periosteum, the stem cell compartment for osteogenesis. Finally, it can be found in kidney and the digestive tract in a subset of tenascin-C-positive regions, but not in the brain.

15 Example 5: Tenascin-W expression in tumour cells

Tenascin-W expression in tumour cells was tested and compared with the known results for tenascin-C which was found to be highly expressed in tumour tissues (Chiu, J. C. & Ghosh, P. K. et al. (1993) *Sem. Cancer Biol.* **4**, 301-310).

Therefore, mouse mammary tumours were used. Mouse mammary tumours
20 develop readily in transgenic mice expressing oncogenes under the control of mammary gland-specific promoters. Overexpression of c-myc results in the growth of non-metastatic tumours whereas overexpression of Ha-ras leads to the development of metastatic tumours (Li, F. et al. (1994) *Int. J. Cancer* **59**, 560-568). Using this model system, high expression of the large variant of
25 tenascin-W (about 190 kDa) was found in the tumours of ras-transgenic mice, but not in the myc- or neuT-transformed non-metastatic tumours, whereas tenascin-C was over-expressed in both types of tumours (see example 2). In transgenic mice overexpressing neuT develop non-metastasizing mammary tumours, whereas in double transgenic mice overexpressing neuT together
30 with EphB4 receptor tyrosine kinase the tumours are metastatic (Munarini, N. et al. (2002) *Cell Sci.* **115**, 25-37). Using this model system we again found high expression of tenascin-W in the metastatic tumours, but not in the non-metastatic ones. These expression patterns were confirmed by SDS-PAGE (SDS-polyacrylamide gel electrophoresis), by fractionating tumour extracts,

blotting on polyvinylidene difluoride membranes, and analyzing the extract using an anti-tenascin-W antisera.

Example 6: Blood serum analysis

5 The content of tenascin-W in serum is analyzed by Western blotting as described for the tissue extracts. For improved sensitivity a Sandwich ELISA test is developed as described previously for tenascin-C (Schenk et al. 1995. Int. J. Cancer 61:443-449). Briefly, 96-well plates are coated with either polyclonal or monoclonal anti-tenascin-W antibodies. Then the serum

10 samples are applied, the wells washed and the bound tenascin-W is detected by either a polyclonal or a monoclonal anti-tenascin-W antibody followed by an appropriate peroxidase-labeled secondary antibody.

Example 7: Adhesion assay

15 The purified tenascin-W was used for cell adhesion studies of MDA-MB435 mammary carcinoma cells, T98G glioblastoma cells and NIH-3T3 fibroblasts. Cells were adhered to tenascin-W coated at 2-100 µg/ml (no adhesion under 2 µg/ml) to a similar extent as to fibronectin, whereas cell adhesion to tenascin-W was minimal. The adhesion to tenascin-W could be blocked by including 20 10 µg/ml of the function blocking anti-β1 integrin P4C10 (Sigma) in the adhesion assay implying a β1 integrin as the cellular receptor for tenascin-W. In general, 60-well microtiter plates (Nunc) were coated with 2-100 µg/ml tenascin-W for 1h at 37°C. The non-coated plastic surface was blocked with 1% heat-inactivated BSA in PBS. Cells were trypsinised, trypsin was blocked 25 with 100 µg/ml soybean trypsin inhibitor in PBS and, cells were resuspended in serum-free medium and counted. 200-500 cells per well were plated for the indicated time points, fixed by addition of glutaraldehyde (2% final concentration) for 15 minutes and stained with 0.1% crystalviolet in 20% methanol for 30 minutes. Cells were observed under a Nikon microscope 30 (Nikon diaphot) and pictures were taken with a Nikon camera.

Example 8: DNA replication and proliferation assay

96-well plates (Falcon) are coated as described above. Cells are serum starved overnight and uncoated wells are coated with 10 µg/ml tenascin-W.

the coated plates in the presence of 1 % serum or 40nM PDGF BB (Platelet-derived growth factor BB). 14h later cells are labelled with radioactive ^3H -thymidine (0.5 $\mu\text{Ci}/\text{well}$) for 4h at 37°C, incorporated ^3H -thymidine precipitated with 10% TCA and determined with a Beckman scintillation counter after cell

5 lysis in 0.3N NaOH, 2% SDS.

Example 9: In vitro binding assay (ELISA)

96-well ELISA plates are coated with the indicated ECM proteins (e.g. fibronectin or tenascin-W for 1h at 37°C, blocked with 1% milkpowder, 0.05%

10 Tween-20 in PBS. ECM proteins (tenascin-W or fibronectin) are added in blocking solution for 1h, washed with blocking solution and the appropriate antibodies are added. Bound proteins are detected by immune reaction with a peroxidase-coupled secondary antibody followed by colour reaction with 21mg/ml citric acid 1-hydrate, 34mg/ml $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 0.4mg/ml

15 phenylenediamine, 1 μl H_2O_2 that is stopped with 4M sulphuric acid. The absorbance was read at 590nm.

Example 10: Immunofluorescence microscopy

10 4 cells are transferred onto 4-well Cellstar plastic plates (Greiner) that are

20 coated with ECM proteins. Cells are fixed with 4% paraformaldehyde, 50mM phosphate buffer, 5mM EDTA in PBS for 15 minutes, blocked with 3% BSA, 0.5% Tween-20 in PBS and incubated with primary and secondary antibodies in blocking solution. Slides are embedded in 10.5% Mowiol containing 2.5% DABCO as antifade agent. Cells are analysed by microscopy.

25 As is apparent to one of ordinary skill in the art, variations in the above-described methods can be introduced with ease to attain the same objective. Various incubating conditions, labels, apparatus and materials can be chosen according to individual preference. All publications referred to herein are

30 incorporated by reference in their entirety as if each were referred to individually.

SEQUENCE LISTING

5 <110> Novartis Forschungsstiftung, Zweigniederlassung Friedrich Miescher
 Institute for Biomedical Research
 10 <120> Tenascin-W Compositions and Uses thereof
 15 <130> 1-32411/P1/FMI
 20 <160> 16
 25 <170> PatentIn version 3.1
 30 <210> 1
 35 <211> 3891
 40 <212> DNA
 45 <213> Mus musculus
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 80 Met Gly Leu Trp Gly Met Leu Ala Phe Pro Leu Gly Phe Pro Leu Ala
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 90 tct gtg ctc ctg gtg gct tcg gcc cca gcc act cca gag tct ccc ggc 96
 95 30 Ser Val Leu Leu Val Ala Ser Ala Pro Ala Thr Pro Glu Ser Pro Gly
 100 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
 105 tgc agc aac aaa gag caa cag gtc act gtt agc cac acc tac aag att 144
 110 35 Cys Ser Asn Lys Glu Gln Gln Val Thr Val Ser His Thr Tyr Lys Ile
 115 40 45 50 55 60 65 70 75 80 85 90 95 100
 120 gac gtg ccc aag tct gct ctg gtt caa gta gag acc gca cag tca 192
 125 40 Asp Val Pro Lys Ser Ala Leu Val Gln Val Glu Thr Asp Pro Gln Ser
 130 50 55 60 65 70 75 80 85 90 95 100
 135 40 ctc agc gat gat ggg aca tca ctc ttg gct ccc ggg gag gat ggg gag 240
 140 55 Leu Ser Asp Asp Gly Thr Ser Leu Leu Ala Pro Gly Glu Asp Gly Glu
 145 60 65 70 75 80 85 90 95 100
 150 45 gag cag aac att atc ttc agg cac aac atc cgt ctt cag aca ccg cag 288
 155 50 Glu Ala Leu Ala Leu Ala Ser Ala Ser Ala Pro Ala Pro Gly Glu

85

90

95

5	aag aat tgc gac ctg gca gac agt gtc cag gac ctg cta gcc cg ^g atg Lys Asn Cys Asp Leu Ala Asp Ser Val Gl ⁿ Asp Leu Leu Ala Arg Met 100	105	110	336
10	aaa aag ctg gag gaa gag atg gca gag ctg aag gag cag tgc aat acc Lys Lys Leu Glu Glu Glu Met Ala Glu Leu Lys Glu Gl ⁿ Cys Asn Thr 115	120	125	384
15	aac cgc tgc tgc cag ggg gct gct gat ctg agc cgt cac tgc agt ggc Asn Arg Cys Cys Gl ⁿ Gly Ala Ala Asp Leu Ser Arg His Cys Ser Gly 130	135	140	432
20	cac ggg acc ttc ctc cct gag acc tgc agc tgc cac tgt gac cag ggc His Gly Thr Phe Leu Pro Glu Thr Cys Ser Cys His Cys Asp Gl ⁿ Gly 145	150	155	480
25	tgg gag ggc gca gac tgt gat cag ccc acc tgt cct ggg gct tgc aac Trp Glu Gly Ala Asp Cys Asp Gl ⁿ Pro Thr Cys Pro Gly Ala Cys Asn 165	170	175	528
30	ggc cac ggg cgc tgt gtg gat ggg ccc tgc tgt gac ggc ccc tat Gly His Gly Arg Cys Val Pro Asp Cys Asp Gl ⁿ Cys Ser Cys Asp Ala Pro Tyr 180	185	190	576
35	gtg ggg gtc gac tgc gcc tac gcc gcc tgt ccc cag gac tgc agt ggg Val Gl ^y Val Asp Cys Ala Tyr Ala Ala Cys Pro Gl ⁿ Asp Cys Ser Gly 195	200	205	624
40	cat ggc gtg tgc gtg cag ggt gtc tgc cag tgc cac gag gac ttc aca His Gly Val Cys Val Gl ⁿ Gly Val Cys Gl ⁿ Cys His Glu Asp Phe Thr 210	215	220	672
45	gca gag gac tgc agc gag cag cgc tgt cct ggc gac tgt agt ggc aat Ala Glu Asp Cys Ser Glu Gl ⁿ Arg Cys Pro Gly Asp Cys Ser Gly Asn 225	230	235	720
	ggt ttc tgt gac act ggc gag tgt tac tgt gag atg ggc ttt act ggc Gly Phe Cys Asp Thr Gly Glu Cys Tyr Cys Glu Met Gly Phe Thr Gly 245	250	255	768
	ccc gac tgt tcc cag gtg gtg gct cct cag ggc ctg cag ttg ctc aag Pro Asp Cys Ser Gl ⁿ Val Val Ala Pro Gl ⁿ Gly Leu Gl ⁿ Leu Leu Lys 260	265	270	816

	agc acg gag aac tct ctg ctg gtg agt tgg gag ccc tcc agt gag gta		864
	Ser Thr Glu Asn Ser Leu Leu Val Ser Trp Glu Pro Ser Ser Glu Val		
	275	280	285
5	gac tac tac ctg ctc agc tac tac ccc ctg ggg aag gag caa gct aca		912
	Asp Tyr Tyr Leu Leu Ser Tyr Tyr Pro Leu Gly Lys Glu Gln Ala Thr		
	290	295	300
10	aaa cag gtc cgg gta ccc aag gag cag cac acc tat gac atc acc ggc		960
	Lys Gln Val Arg Val Pro Lys Glu Gln His Thr Tyr Asp Ile Thr Gly		
	305	310	315
	320		
15	ttg ctg cct gga acc aag tac ata gtc acc ctg cgc aac gtg aag aaa		1008
	Leu Leu Pro Gly Thr Lys Tyr Ile Val Thr Leu Arg Asn Val Lys Lys		
	325	330	335
	gac att tcc agc agc cct cag cat cta ctt gcc acc aca gac ctt gct		1056
	Asp Ile Ser Ser Ser Pro Gln His Leu Leu Ala Thr Thr Asp Leu Ala		
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	Val Leu Gly Thr Ala Trp Val Asn Glu Glu Thr Glu Thr Ser Leu Asp		
	355	360	365
25	gtg ggg tgg ggg aat ctt ctg act gag gtg gac tat tac aag ctt cgg		1152
	Val Glu Glu Glu Asn Pro Leu Thr Glu Val Asp Tyr Tyr Lys Leu Arg		
	370	375	380
30	tat ggc ccc tta aca ggg cag gag gtg aca gag gtc act gtg ccc aag		1200
	Tyr Gly Pro Leu Thr Gly Gln Glu Val Thr Glu Val Thr Val Pro Lys		
	385	390	395
	400		
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	Ser Arg Asp Pro Lys Ser Arg Tyr Asp Ile Thr Gly Leu Gln Pro Gly		
	405	410	415
	420	425	430
40	acg gaa tat aaa atc aca gtt gtg ccc atc cga ggt gat ctg gag gga		1296
	Thr Glu Tyr Lys Ile Thr Val Val Pro Ile Arg Gly Asp Leu Glu Gly		
	440	445	
45	aag ccg att ctc ctg aat ggc agg aca gaa att gat gga cca acc aat		1344
	Lys Pro Ile Leu Leu Asn Gly Arg Thr Glu Ile Asp Gly Pro Thr Asn		
	435	440	445
	450		
	gtg gtc aca aat cag gtg aca gaa gac aca gca tct gtt tcc tgg gat		1392
	Val Val Cys Trp Asn Val Thr Glu Asp Pro Ala Cys Val Val Thr Gln		

	450	455	460	
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	gat ggg gag acc aag gag aag gca gta cca aag gac cag agc agc acc Asp Gly Glu Thr Lys Glu Lys Ala Val Pro Lys Asp Gln Ser Ser Thr			1488
	485	490		495
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	500	505		510
15	gct gag agg ggc aac caa ggc agc aag aaa gca gac acc aag gcc ctc Ala Glu Arg Gly Asn Gln Gly Ser Lys Lys Ala Asp Thr Lys Ala Leu			1584
	515	520		525
20	aca gaa att gac agt cca gaa aac ctg gtg act gac cgg gtg aca gag Thr Glu Ile Asp Ser Pro Glu Asn Leu Val Thr Asp Arg Val Thr Glu			1632
	530	535		540
25	aac agc ctc tct gtc tcg tgg gac cca gtg gag gct gac atc gac agg Asn Ser Leu Ser Val Ser Trp Asp Pro Val Glu Ala Asp Ile Asp Arg			1680
	545	550		555
				560
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	565	570		575
35	gtg aag aag gac cag agg agc acc gtc ctc acc ggc ctg agt ccc ggt Val Lys Lys Asp Gln Arg Ser Thr Val Leu Thr Gly Leu Ser Pro Gly			1776
	580	585		590
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	595	600		605
45	aag aag gcc aac acc aag gct ccc aca gac atc gac agc ccc aaa aac Lys Lys Ala Asn Thr Lys Ala Pro Thr Asp Ile Asp Ser Pro Lys Asn			1872
	610	615		620
	ttg gta act gac cag gtg aca gag aac act ctc agt gtc tcc tgg gac Leu Val Thr Asp Gln Val Thr Glu Asn Thr Leu Ser Val Ser Trp Asp			1920
	625	630		640

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20	acc act ctt agt gtc tcc tgg gac cca gtg gag gct gac att gat agg	2208		
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	725	730	735	
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	cta gtg act gag cag gtg gca gag agc act gcc acc gtg tcc tgg gac	2448		
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45	cca gta gag gct gac atc gac agg tat gtg gtg cgc tac acc tct gct	2496		
	Arg Lys Cta Ala Asp Thr Asp Arg Val Val Val Thr Thr Ser Ala			

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	Asp Gly Glu Thr Arg Glu Ile Pro Val Arg Lys Glu Lys Ser Ser Thr			
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	Val Leu Thr Gly Leu Arg Pro Gly Val Glu Tyr Thr Val Gln Val Trp			
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	Ala Gln Lys Gly Ala Arg Glu Ser Lys Lys Ala Lys Thr Lys Ala Pro			
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	Thr Glu Ile Asp Ser Pro Lys Asn Leu Val Thr Asn Arg Val Thr Glu			
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	Asn Thr Ala Thr Ile Ser Trp Asp Pro Val Arg Ala Asn Ile Asp Arg			
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	Tyr Met Val Arg Tyr Thr Ser Ala Asp Gly Glu Thr Lys Glu Ile Pro			
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	Val Ser Lys Asp Gln Ser Asn Thr Ile Leu Thr Gly Leu Lys Pro Gly			
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	Met Glu Tyr Thr Ile His Val Trp Ala Gln Lys Gly Ala Arg Glu Ser			
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35	aag aag gct gat acc aag gcc cta aca gaa att gac cct ccc aga aat			2928
	Lys Lys Ala Asp Thr Lys Ala Leu Thr Glu Ile Asp Pro Pro Arg Asn			
	965	970	975	
40	ctc cgt ccg ttc ggg gta aca cat tct ggt ggg gtt ttg acc tgg ttg			2976
	Leu Arg Pro Phe Gly Val Thr His Ser Gly Gly Val Leu Thr Trp Leu			
	980	985	990	
	ccc cca tct gct caa att gat ggc tac att ttg acc tac cag ttc cca			3024
	Pro Pro Ser Ala Gln Ile Asp Gly Tyr Ile Leu Thr Tyr Gln Phe Pro			
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	Thr Leu Ser Thr Val Asp Ala Arg Phe Pro His Pro Ser Asp Cys		
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	Ser Gln Val Gln Gln Asn Thr Asn Ala Ala Ser Gly Leu Tyr Thr		
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	Asp Met Asp Asp Gly Gly Gly Trp Ile Val Phe Gln Arg Arg		
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	Asn Thr Gly Gln Leu Asp Phe Phe Lys Arg Trp Arg Ser Tyr Val		
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	Glu Gly Phe Gly Asp Pro Met Lys Glu Phe Trp Leu Gly Leu Asp		
	1130 1135 1140		
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	Lys Leu His Asn Leu Thr Thr Gly Thr Thr Arg Tyr Glu Val		
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	Asn Cys His Leu Ala Asn Pro	Asn Gly Lys Tyr Gly	Glu Thr Lys		
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	His Ser Glu Gly Val Asn Trp	Glu Pro Trp Lys Gly	His Glu Phe		
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	Arg Asp Arg Phe Ser Gly Arg	Lys Lys Arg Ser Ile	Gly Lys Ala		
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25

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	Asp Val Pro Lys Ser Ala Leu Val Gln Val Glu Thr Asp Pro Gln Ser			
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	Glu Gln Asn Ile Ile Phe Arg His Asn Ile Arg Leu Gln Thr Pro Gln			
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15	Lys Asn Cys Asp Leu Ala Asp Ser Val Gln Asp Leu Leu Ala Arg Met			
	100	105	110	
	Lys Lys Leu Glu Glu Glu Met Ala Glu Leu Lys Glu Gln Cys Asn Thr			
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	Asn Arg Cys Cys Gln Gly Ala Ala Asp Leu Ser Arg His Cys Ser Gly			
	130	135	140	
25	His Gly Thr Phe Leu Pro Glu Thr Cys Ser Cys His Cys Asp Gly			
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	Trp Glu Gly Ala Asp Cys Asp Gln Pro Thr Cys Pro Gly Ala Cys Asn			
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30	Gly His Gly Arg Cys Val Asp Gly Gln Cys Val Cys Asp Ala Pro Tyr			
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	Gly Phe Cys Asp Thr Gly Glu Cys Tyr Cys Glu Met Gly Phe Thr Gly			
	245	250	255	
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Tyr Gly Pro Leu Thr Gly Gln Glu Val Val Val Thr Val Pro Lys
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Val Leu Thr Gly Leu Lys Pro Gly Glu Ala Tyr Lys Val Phe Val Trp
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Lys Lys Ala Asp Thr Lys Ala Leu Thr Glu Ile Asp Pro Pro Arg Asn
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Pro Pro Ser Ala Gln Ile Asp Gly Tyr Ile Leu Thr Tyr Gln Phe Pro
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	Glu Met Ala Val His Lys Asp Glu Ser Ser Thr Val Leu Thr Gly Leu		
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	Lys Pro Gly Glu Ala Tyr Lys Val Tyr Val Trp Ala Glu Arg Gly Asn		
	500	505	510
25	cag ggg agc aag aaa gct gac acc aat gcc ctc aca gaa att gac		1584
	Gln Gly Ser Lys Ala Asp Thr Asn Ala Leu Thr Glu Ile Asp Ser		
	515	520	525
30	cca gca aac ctg gtg act gac cgg gtg act gag aat acc gcc acc atc		1632
	Pro Ala Asn Leu Val Thr Asp Arg Val Thr Glu Asn Thr Ala Thr Ile		
	530	535	540
35	tcc tgg gac ccg gta cag gcc acc att gac aag tac gtg gtg cgc tac		1680
	Ser Trp Asp Pro Val Gln Ala Thr Ile Asp Lys Tyr Val Val Arg Tyr		
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	560		
	acc tct gct gac gac caa gag acc aga gag gtt ctg gtg ggg aag gag		1728
	Thr Ser Ala Asp Asp Gln Glu Thr Arg Glu Val Leu Val Gly Lys Glu		
	565	570	575
40	cag agc agc act gtc ctg aca ggc ctg agg cca ggt gtg gag tac aca		1776
	Gln Ser Ser Thr Val Leu Thr Gly Leu Arg Pro Gly Val Glu Tyr Thr		
	580	585	590
45	gtg cat gtc tgg gcc cag aag ggg gac cga gag agc aag aag gct gac		1824
	Val His Val Arg Gln Gln Lys Glu Asp Arg Gln Ser Thr Asp Val Asp		

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	acc aac gcc ccg aca gat att gac agc ccc aaa aac ctg gtg act gac			1872
	Thr Asn Ala Pro Thr Asp Ile Asp Ser Pro Lys Asn Leu Val Thr Asp			
5	610	615	620	
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	Arg Val Thr Glu Asn Met Ala Thr Val Ser Trp Asp Pro Val Gln Ala			
	625	630	635	640
10	gcc att gac aag tac gtg gtg cgc tac acc tct gct ggt gga gag acc			1968
	Ala Ile Asp Lys Tyr Val Val Arg Tyr Thr Ser Ala Gly Gly Glu Thr			
	645	650	655	
15	agg gag gtt ccg gtg ggg aag gag cag agc agc aca gtc ctg aca ggc			2016
	Arg Glu Val Pro Val Gly Lys Glu Gln Ser Ser Thr Val Leu Thr Gly			
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20	ctg aga ccg ggt atg gag tac atg gtg cac gtg tgg gcc cag aag ggg			2064
	Leu Arg Pro Gly Met Glu Tyr Met Val His Val Trp Ala Gln Lys Gly			
	675	680	685	
25	gac cag gag agc aag aag gcc gac acc aag gtc acc att gac			2112
	Asp Gln Glu Ser Lys Lys Ala Asp Thr Lys Asn Gln Thr Asp Ile Asp			
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	Ser Pro Gln Asn Leu Val Thr Asp Arg Val Thr Glu Asn Met Ala Thr			
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	Val Ser Trp Asp Pro Val Arg Ala Thr Ile Asp Arg Tyr Val Val Arg			
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	Tyr Thr Ser Ala Lys Asp Gly Glu Thr Arg Glu Val Pro Val Gly Lys			
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	Glu Gln Ser Ser Thr Val Leu Thr Gly Leu Arg Pro Gly Val Glu Tyr			
	755	760	765	
45	acg gtg cac gtg tgg gcc cag aag ggg gcc cag gag agc aag aag gct			2352
	Thr Val His Val Trp Ala Gln Lys Gly Ala Gln Glu Ser Lys Lys Ala			
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gac acc aag gcc cag aca gac att gac agc ccc caa aac ctg gtc act			2400
Asp Thr Lys Ala Gln Thr Asp Ile Asp Ser Pro Gln Asn Leu Val Thr			
785	790	795	800
5 gac tgg gtg aca gag aat aca gcc act gtc tcc tgg gac ccg gtg cag			2448
Asp Trp Val Thr Glu Asn Thr Ala Thr Val Ser Trp Asp Pro Val Gln			
805	810	815	
10 gcc acc att gac agg tat gtg gtg cac tac acg tct gcc aac ggaa gag			2496
Ala Thr Ile Asp Arg Tyr Val Val His Tyr Thr Ser Ala Asn Gly Glu			
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15 acc agg gag gtt cca gtg ggg aag gag cag agc agc act gtc ctg acg			2544
Thr Arg Glu Val Pro Val Gly Lys Glu Gln Ser Ser Thr Val Leu Thr			
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20 ggc ctg agg ccg ggc atg gag tac acg gtg cac gtg tgg gcc cag aag			2592
Gly Leu Arg Pro Gly Met Glu Tyr Thr Val His Val Trp Ala Gln Lys			
850	855	860	
25 ggg aac cag gag agc aag aag gct gac acc aag gcc cag aca gaa att			2640
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30 act gtc tcc tgg gac ccg gtt cag gcc acc att gac aag tac atg gtg			2688
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Arg Tyr Thr Ser Ala Asp Gly Glu Thr Arg Glu Val Pro Val Gly Lys			
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40 gag cac agc agc act gtc ctg acg ggc ctg aga cca ggc atg gag tac			2784
Glu His Ser Ser Thr Val Leu Thr Gly Leu Arg Pro Gly Met Glu Tyr			
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45 atg gtg cac gtg tgg gcc cag aag ggg gcc cag gag agc aag aag gct			2832
Met Val His Val Trp Ala Gln Lys Gly Ala Gln Glu Ser Lys Lys Ala			
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			2928

965

970

975

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	Val Lys Glu Met Gln Leu Gly Arg Glu Asp Gln Arg Phe Ala Leu		
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	Thr Val Gln Ala Arg Phe Pro His Pro Ser Asp Cys Ser Gln Val		
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	Gln Gln Asn Ser Asn Ala Ala Ser Gly Leu Tyr Thr Ile Tyr Leu		
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	His Gly Asp Ala Ser Arg Pro Leu Gln Val Tyr Cys Asp Met Glu		
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35	acg gac gga ggt ggc tgg att gtc ttc cag agg cgg aac act ggg	3339	
	Thr Asp Gly Gly Gly Trp Ile Val Phe Gln Arg Arg Asn Thr Gly		
	1100	1105	1110
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	Gln Leu Asp Phe Phe Lys Arg Trp Arg Ser Tyr Val Glu Gly Phe		
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	Tyr Arg Gly Thr Ala Gly Asp	Ala Leu Thr Tyr His	Asn Gly Trp	
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	Lys Phe Thr Thr Phe Asp Arg	Asp Asn Asp Ile Ala	Leu Ser Asn	
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	Cys Ala Leu Thr His His Gly	Gly Trp Trp Tyr Lys	Asn Cys His	
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25	ttg gcc aac cct aat ggc aga	tat ggg gag acc aag	cac agt gag	3744
	Leu Ala Asn Pro Asn Gly Arg	Tyr Gly Glu Thr Lys	His Ser Glu	
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30	ggg gtg aac tgg gag cct tgg	aaa gga cat gaa ttc	tcc att cct	3789
	Gly Val Asn Trp Glu Pro Trp	Lys Gly His Glu Phe	Ser Ile Pro	
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35	tac gtg gag ttg aaa atc cgc	cct cat ggc tac agc	agg gag cct	3834
	Tyr Val Glu Leu Lys Ile Arg	Pro His Gly Tyr Ser	Arg Glu Pro	
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20 Pro Lys Glu Gln His Ser Tyr Glu Ile Leu Gly Leu Leu Pro Gly Thr
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Lys Tyr Ile Val Thr Leu Arg Asn Val Lys Asn Glu Val Ser Ser Ser
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25 Pro Gln His Leu Leu Ala Thr Val Leu Ala Val Leu Gly Thr Ala
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Trp Val Thr Asp Glu Thr Glu Asn Ser Leu Asp Val Glu Trp Glu Asn
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Pro Ser Thr Glu Val Asp Tyr Tyr Lys Leu Arg Tyr Gly Pro Met Thr
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35 Gly Gln Glu Val Ala Glu Val Thr Val Pro Lys Ser Ser Asp Pro Lys
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Ser Arg Tyr Asp Ile Thr Gly Leu His Pro Gly Thr Glu Tyr Lys Ile
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Asp Gln Glu Ser Lys Lys Ala Asp Thr Lys Ala Gln Thr Asp Ile Asp
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Ser Pro Gln Asn Leu Val Thr Asp Arg Val Thr Glu Asn Met Ala Thr
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Tyr Thr Ser Ala Lys Asp Gly Glu Thr Arg Glu Val Pro Val Gly Lys
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Glu His Ser Ser Thr Val Leu Thr Gly Leu Arg Pro Gly Met Glu Tyr
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10 Lys Phe Thr Thr Phe Asp Arg Asp Asn Asp Ile Ala Leu Ser Asn
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15 Leu Ala Asn Pro Asn Gly Arg Tyr Gly Glu Thr Lys His Ser Glu
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20 Gly Val Asn Trp Glu Pro Trp Lys Gly His Glu Phe Ser Ile Pro
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Tyr Val Glu Leu Lys Ile Arg Pro His Gly Tyr Ser Arg Glu Pro
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Phe

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CLAIMS:

1. A composition comprising an isolated nucleic acid molecule having a nucleotide sequence selected from the group consisting of:

- 5 (a) a nucleotide sequence as set forth in SEQ ID NO: 1;
- (b) a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 2
- (c) a nucleotide sequence with at least 85% identity to the sequence of (a) or (b);
- 10 (d) a subsequence of at least 50 consecutive nucleotides of a sequence of (a), (b) or (c), with the proviso that said subsequence does not fall between nucleotide 1027 and nucleotide 1076 of SEQ ID NO: 1; and
- (e) a nucleotide sequence complementary to any of the nucleotide sequences in (a),(b), (c), or (d).

2. The composition of claim 1, said composition comprising said isolated nucleic acid molecule having said nucleotide sequence with at least 85% identity to the sequence of (a) and encoding a variant of the amino acid sequence shown in SEQ ID NO: 2.

3. The composition of claim 2, wherein said variant comprises a conservative substitution of at least one amino acid in said amino acid sequence in SEQ ID: NO: 2.

- 25 4. The composition of claim 3, wherein said variant has stem cell differentiation inducing activity.
- 5. The composition of claim 1, said composition comprising said isolated nucleic acid molecule encoding the amino acid sequence shown in SEQ ID NO: 2.

6. The composition of claim 1, wherein said composition comprises a nucleic acid molecule having a subsequence that is antisense to SEQ ID NO:1 or a sequence having at least 85% identity thereto.
- 5 7. The composition of claim 6, wherein said antisense nucleic acid molecule comprises nucleotide residues that are resistant to nuclease degradation.
8. A nucleic acid vector comprising the nucleic acid molecule of any one 10 of claims 1 to 5.
9. A host cell comprising the vector of claim 8.
10. A composition comprising an isolated polypeptide having an amino acid sequence selected from the group consisting of:
 - 15 (a) an amino acid sequence as set forth in SEQ ID No. 2; and
 - (b) an amino acid sequence with at least 85% identity to the sequence of (a); and
 - a subsequence of at least 30 consecutive amino acids of the sequence of (a) or (b), with the proviso that said 20 subsequence does not fall within amino acid nos.1102 and 1152 of SEQ ID NO:2.
11. The composition of claim 10, wherein said amino acid sequence in (b) 25 comprises a conservative substitution of at least one amino acid in said amino acid sequence of SEQ ID: NO: 2.
12. The composition of claim 10, wherein said polypeptide or fragment has stem cell differentiation inducing activity.
- 30 13. The composition of claim 10, said composition comprising said fragment, said fragment exhibiting an epitope recognized by polyclonal antibodies raised against the polypeptide having the amino acid sequence shown in SEQ ID NO: 2.

14. The composition of claim 10, said composition comprising said polypeptide encoding the amino acid sequence shown in SEQ ID NO: 2.

5

15. An antibody specifically reactive against the composition of any one of claims 10 to 12, or 14

10 16. A composition comprising an isolated nucleic acid molecule having a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence as set forth in SEQ ID No. 1 or SEQ ID No. 3;
- (b) a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 2 or SEQ ID NO: 4;
- (c) a nucleotide sequence with at least 35% identity to any one of the sequences of (a) or (b), preferably (a);
- (d) a subsequence of a least 15 consecutive nucleotides of the sequence of (a),(b) or (c); and
- (e) a nucleotide sequence complementary to (a),(b), (c), or (d),

20

and a pharmaceutically acceptable excipient, diluent or carrier.

17. The composition of claim 16, wherein said nucleic acid molecule encodes a protein having stem cell differentiation inducing activity.

25

18. The composition of claim 16, wherein said nucleic acid molecule has a subsequence that is antisense to SEQ ID NO:1 or SEQ ID NO:3.

30 19. The composition of claim 18, wherein said antisense nucleic acid molecule comprises nucleotide residues that are resistant to nuclease degradation.

20. The composition of claim 16, said composition comprising said isolated nucleic acid molecule encoding the amino acid sequence shown in SEQ ID NO: 2 or SEQ ID NO:4.

5 21. A composition comprising an isolated nucleic acid molecule as claimed in claim 16 for use as a pharmaceutical.

22. The composition of claim 21, wherein said isolated nucleic acid molecule has a subsequence that is antisense to SEQ ID NO:1 or SEQ 10 ID NO:3.

15 23. A composition as claimed in any of claims 16 or 17, comprising said nucleic acid molecule having a subsequence selected from the group consisting of nucleotides 2380-3171 of SEQ ID No:1, nucleotides 2371-3162 of SEQ ID No:3, a complement of nucleotides 2380-3171 of SEQ ID No:1, and a complement of nucleotides 2371-3162 of SEQ ID No:3, or an RNA equivalent thereof.

20 24. The use of the nucleic acid molecule of any of claims 16 or 17 for the manufacture of a medicament for the prophylaxis or treatment of cancer or bone pathologies.

25 25. A composition comprising tenascin-W and a pharmaceutically acceptable excipient, diluent or carrier.

26. The composition of claim 25, wherein said tenascin-W is recombinant.

27. A composition as claimed in claim 25, wherein said tenascin-W is a polypeptide having an amino acid sequence selected from the group 30 consisting of:

(a) an amino acid sequence as set forth in SEQ ID No. 2 or 4;

(b) an amino acid sequence with at least 35% identity to the sequence of (a); and

(c) a subsequence of at least 30 consecutive amino acids of the sequence of (a) or (b).,

28. The composition of claim 27, wherein said polypeptide has stem cell differentiation inducing activity.

5 29. The composition of claim 28, said composition comprising said polypeptide encoding the amino acid sequence shown in SEQ ID NO: 4.

10 30. The use of tenascin-W for the treatment or prophylactic treatment of any disease or condition requiring increased tenascin-W levels, e.g. thrombosis, wound healing or atherosclerosis, or for the treatment or prophylactic treatment of a condition ameliorated by the promotion of osteogenesis, e.g. bone healing, osteoporosis.

15 31. The use as claimed in claim 30, wherein tenascin-W has any one or more of the features of the composition(s) of claims 27 to 29.

20 32. The use of tenascin-W as a stem cell marker.

33. An antibody that specifically recognizes tenascin W for use as pharmaceutical.

25 34. The use of an antibody that specifically recognizes tenascin W for the manufacture of a medicament, for the prophylaxis or treatment of cancer, or for the prophylaxis or treatment of any disease or condition involving tenascin-W, e.g. excessive bone growth.

30 35. The use as claimed in claim 34, wherein said cancer is a metastatic tumour.

36. The use as claimed in any of claims 34 to 35, wherein the metastatic

37. The use as claimed in any of claims 34 to 36, wherein the tumour is a solid tumour.

5 38. The use as claimed in any of claims 34 to 37, wherein the tumour is a glioblastoma, prostate, lung, colorectal, osteo- or breast carcinoma.

10 39. A method for identifying agents for the prevention or the prophylactic treatment of tumourigenesis or the treatment or prophylactic treatment of tumours or cancer, or the treatment or prophylactic treatment of any disease or condition involving tenascin-W, e.g. a condition ameliorated by the promotion of osteogenesis, comprising contacting a test compound with a tenascin-W expressing cell sample and then measuring a change in one or more of:

15 (a) cell proliferation, e.g. cell progression entering S-phase of the cell cycle;

 (b) DNA synthesis;

 (c) cell adhesion;

 (d) cell spreading;

20 (e) focal adhesion and actin stress fibre formation on fibronectin;

 (f) cell binding to extracellular matrix (ECM)

relative to when said test compound is absent.

25 40. A method as claimed in claim 39, further comprising measuring a change in tenascin-W expression relative to when said test compound is absent.

30 41. A method as claimed in any one of the claims 39 or 40, wherein tenascin-W has any one or more of the features of the compositions of claims 27 to 29.

42. A method as claimed in any of claims 39 to 41, comprising culturing cells on a substrate.

43. A method as claimed in any of claims 39 to 42, wherein said sample is a human sample.

5 44. A method as claimed in any of claims 39 to 43, wherein said sample is a tissue sample.

45. A method as claimed in any of claims 39 to 43, wherein said sample is blood serum.

10 46. A method as claimed in any of claims 39 to 43, wherein said sample is cell free sample.

47. A method as claimed in claim 46 comprising measuring the binding of a test compound to tenascin-W, optionally also comprising measuring the binding of a control compound to tenascin-W.

15 48. A method as claimed in any of claims 39 to 47, wherein tenascin-W is measured using an antibody reactive against tenascin-W.

20 49. A method as claimed in claim 48, wherein said antibody is labelled with a fluorescent label, a fluorescence quencher, a radioactive label, a scintillant or an enzyme.

25 50. A method as claimed in any of claims 39 to 48, wherein tenascin-W is attached to a solid surface.

51. A method as claimed in claim 50 in the form of an enzyme linked immunosorbent assay (ELISA).

30 52. A substance for the prevention or the prophylactic treatment of tumourigenesis or the diagnosis or the treatment or prophylactic treatment of tumours, or the treatment or prophylactic treatment of any disease or condition involving tenascin-W and/or tenascin-W receptor.

by the promotion of osteogenesis, identified by a method as claimed in any of claims 39 to 51.

53. A method of diagnosing or prognosing cancer comprising:

- 5 (a) obtaining a sample from an individual;
- (b) analysing said sample for the presence of tenascin-W
- (c) correlating the presence of tenascin-W with an unfavourable prognosis or diagnosis.

10 54. A method of diagnosing or prognosing cancer as claimed in claim 53, comprising correlating in (c) an increase in tenascin-W relative to healthy tissue with an unfavourable prognosis or diagnosis.

15 55. A method of diagnosing or prognosing cancer as claimed in any of claims 53 to 54, wherein said sample is blood serum from an individual.

20 56. A method of diagnosing or prognosing cancer as claimed in any of claims 53 to 55, further comprising propagating cells in said sample by cell culture.

25 57. A method of diagnosing or prognosing cancer as claimed in any of claims 53 to 56, wherein said tenascin-W is detected using an antibody specific for tenascin-W, optionally wherein a control antibody specific to another tenascin is used to test for specificity.

30 58. A method of diagnosing or prognosing cancer as claimed in any of claims 53 to 57, wherein said tenascin-W is detected using an antibody specific for tenascin-W expressed in tumour tissue, optionally wherein a control antibody specific to tenascin-W expressed in healthy tissue, e.g. the small variant of tenascin-W (170kD) in blood serum of healthy individual, is used in a control reaction.

ABSTRACTTENASCIN-W COMPOSITIONS AND USES THEREOF

5

Tenascin-W, an extracellular matrix molecule that is specifically expressed in metastatic tumours is provided. A system comprising a sample expressing tenascin-W is used as an *in vitro* method for screening possible anti-tumour agents or for agents that promote osteogenesis. Test substances are

10 assayed for the ability to inhibit expression of tenascin-W in tumour cells or to increase expression of tenascin-W in cells of the periosteum.

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